

Add new claims 159 and 160

D3
-159. The method of claim 157, wherein Fe^{2+} and/or Fe^{3+} is present at a concentration of about 0.00028 to 0.011 g/L, and the concentration of Zn^{2+} is about 0.00007 to 0.00073 g/L.

160. The method of claim 159, wherein the concentration of Fe^{2+} and/or Fe^{3+} is about 0.0011 g/L and the concentration of Zn^{2+} is about 0.000354 g/L.

Remarks

Applicants respectfully request that the Examiner reconsider and withdraw the outstanding rejections.

I. Status of the Claims

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Claims 159 and 160 have been added. Claims 1-37, 73-77, 79-82, 106-112, 140, 143 and 145-160 are active in the present application.

II. Support for the Amendment

Support for the amendment of claims 152 and 153, and for new claims 159 and 160, is found in the specification, for example, at page 40, line 19 through page 41, line 3. A bracketed/underlined version of the amended claims 152, 153, and 157 is attached hereto.

No new matter has been added by this amendment.

III. Priority Under 35 U.S.C. § 119(a)-(d)

A claim to priority under 35 U.S.C. § 119(a)-(d) and a certified copy of the priority document were filed on July 21, 2000. Prompt acknowledgment of this claim and submission is respectfully requested.

IV. Information Disclosure Statements

At page 2 of the Office Action, the Examiner requested that Applicants clarify how many information disclosure statements have been filed in the present application. A total of four information disclosure statement with form 1449 have been filed. An information disclosure statement and form 1449 were filed on August 11, 1998. A first supplemental information disclosure statement and form 1449 were filed on September 24, 1999. A second supplemental information disclosure statement and form 1449 were filed on July 21, 2000. A third supplemental information disclosure statement and form 1449 were filed on July 28, 2000.

The Examiner has made consideration of all documents listed on the submitted forms 1449 of record in the present application. However, it may be that the Examiner's file does not contain a copy of the information disclosure statement filed on August 11, 1998, and a copy of the first supplemental information disclosure statement filed on September 24, 1999. Accordingly, filed herewith are a copy of the information disclosure statement filed on August 11, 1998, a copy of the Form PTO-1449 filed August 11, 1998 and signed by the Examiner on

December 6, 1999, a postcard date-stamped August 11, 1998, a copy of the first supplemental information disclosure statement filed on September 24, 1999, a copy of the Form PTO-1449 filed September 24, 1999 and signed by the Examiner on December 8, 1999, and a postcard date-stamped September 24, 1999.

V. The Rejection Of Claim 157, Under 35 U.S.C. § 112, First Paragraph, Must Be Withdrawn

At page 3 of the Office Action, the Examiner rejected claim 157, under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter that was not enabled by the present specification. Applicants respectfully traverse this rejection.

Claim 157 is directed to a method for replacing protein in a mammalian cell culture medium, the method comprising replacing insulin with a Zn^{2+} salt and/or replacing transferrin with a Fe^{2+} chelate and/or replacing transferrin with a Fe^{3+} chelate.

The Examiner has the burden of establishing, by objective evidence or sound scientific reasoning, why there is reason to doubt that the claimed method is not in compliance with the enablement requirement of 35 U.S.C. § 112, first paragraph. *See In re Corthright*, 49 USPQ2d 1464, 1466 (Fed. Cir. 1999); *In re Marzocchi*, 169 USPQ 367, 369 (CCPA 1971); and MPEP § 2164.04. At page 3 of the Office Action, the Examiner stated:

The method for replacing protein in a mammalian cell culture medium is not well taught in the specification. There would be a certain degree of unpredictability involved with the process of carrying out this method. In the absence of proper guidance it is uncertain that one of skill in the art would [sic] be capable of carrying out the claimed process without undue burden of experimentation since not [sic] exemplified disclosure is set forth

for which to show one of skill how to go about replacing insulin or transferrin with the ionic chelators claimed herein.

Applicants respectfully disagree. The Examiner has provided only conclusory statements that the claimed methods allegedly are not enabled. The Examiner has not provided any evidence or sound scientific reasoning why there is reason to doubt that the claimed methods are not in compliance with the enabling requirement of 35 U.S.C. § 112, first paragraph. Absent such evidence or sound scientific reasoning, this rejection is improper.

Specifically, the Examiner has not provided any evidence or sound scientific reasoning why there is reason to doubt that one of ordinary skill in the art could, after reading the present specification, make a mammalian cell culture medium in which a Zn^{2+} salt was substituted for insulin, and/or in which a Fe^{2+} chelate and/or a Fe^{3+} chelate was substituted for transferrin. At page 40 of the present specification, line 3 to page 41, line 3, Applicants disclose non-limiting examples of Zn^{2+} salts, Fe^{2+} chelates and Fe^{3+} chelates that can be used.

In addition, the Examiner has not established that there would have been a "certain degree of unpredictability" in practicing the method of claim 157. Moreover, even if it had been established that the method of claim 157 were somewhat unpredictable, and it has not been established, it has not been established that such unpredictability would equate with undue experimentation.

Given Applicants' disclosure, and the knowledge possessed by those of ordinary skill in the art when Applicants' application was filed, the method of claim 157 could have been practiced by one of ordinary skill in the art without undue experimentation. This rejection is,

thus, improper. Applicants respectfully request that this rejection be reconsidered and withdrawn.

VI. *The Rejection Of Claims 1-29, 79, 140, 154 and 158 Over Israel Must Be Withdrawn*

At page 4 of the Office Action, the Examiner rejected claims 1-29, 79, 140, 154 and 158, under 35 U.S.C. § 102(b), as allegedly anticipated by Israel, U.S. Patent Number 5,318,898 ("Israel"), or, in the alternative, under 35 U.S.C. § 103(a) as obvious over Israel. Applicants respectfully traverse these rejections.

A. *The Methods Of Claims 1-29, 79, 140, 154 and 158*

Claims 1 and 22 recite methods of cultivating a mammalian cell in suspension *in vitro*, using a medium that is serum-free and chemically defined. Claim 15 recites a method of cultivating a mammalian cell in suspension *in vitro*, using a medium that is chemically defined. Claims 2-14, 79, and 140 depend, either directly or indirectly, from claim 1. Claims 16-21 depend, either directly or indirectly, from claim 15. Claims 23-29 depend, either directly or indirectly, from claim 22.

Claim 154 recites that the chemically defined medium recited in claim 15 is serum-free.

Claim 158 recites a method of cultivating a mammalian cell in suspension *in vitro*, using a serum-free, non-animal derived cell culture medium.

B. Israel Fails To Teach The Methods Of Claims 1-29, 79, 140, 154 and 158

1. Israel Fails To Teach Each Element Of Each Of Claims 1-29, 79, 140, 154 and 158

Israel fails to anticipate claims 1-29, 79, and 140, because Israel fails to teach cultivation of a cell in suspension *in vitro*, using a medium that is chemically defined.

Israel also fails to anticipate claim 6, because Israel fails to teach a medium that is protein-free.

Israel also fails to anticipate claim 7, in which a 1X medium formulation is recited.

Israel also fails to anticipate claim 8, in which a 10X concentrated medium formulation is recited.

Israel also fails to anticipate claims 15, 20, 21 and 27-29, because Israel fails to teach the ingredients recited in claims 15, 20, 21 and 27-29.

Israel also fails to anticipate claim 140, because Israel fails to teach a medium that is free of animal-derived ingredients.

Israel fails to anticipate the method of claim 154, because Israel fails to teach a medium that is both chemically defined and serum-free.

Israel fails to anticipate the method of claim 158, because Israel fails to teach a serum-free medium that free of animal-derived ingredients.

2. Israel Fails To Explicitly Anticipate

At page 4 of the Office Action, the Examiner stated that Israel teaches "suspension culturing of cells in a culture medium containing known ingredients (i.e. chemically defined

culture medium). . . . The claim limitations which are silent in Israel are inherent to the teachings of the cited reference."

Applicants respectfully disagree. Israel fails to teach a chemically defined medium. A medium in which *each* ingredient is known is chemically defined. But, a medium that merely contains known ingredients, as well as unknown ingredients, is not chemically defined. Israel fails to explicitly teach a medium in which each ingredient is known. Thus, Israel fails to explicitly teach a chemically defined medium.

3. Israel Fails To Inherently Anticipate

Israel fails to inherently teach a chemically defined medium. Inherency "may not be established by probabilities or possibilities. The mere fact that a certain thing *may* result from a given set of circumstances is not sufficient." *Continental Can Co. USA, Inc. v. Monsanto Co.*, 20 USPQ2d 1746, 1749 (Fed. Cir. 1991) (citation omitted); *see also* MPEP § 2112 ("The fact that a certain result or characteristic *may* occur or be present in the prior art is not sufficient to establish the inherency of that result or characteristic."). At best, Israel discloses:

The mammalian cells may be grown in any suitable medium, such as α -MEM, Dulbecco's MEM, RPMI 1640, and other media (Freshney, R.I., *Culture of Animal Cells, A Manual of Basic Technique*, Alan R. Liss, Inc., New York (1983)). The cells may be grown in the presence or absence of a serum supplement such as fetal bovine serum (FBS).

Israel at column 1, lines 37-43. Israel also discloses that "[t]he addition of FBS is not necessary for the practice of the present invention." *See* Israel at column 1, lines 56-58.

The disclosure by Israel that cells be grown in the "absence of a serum supplement" and that "FBS is not necessary" does not inherently teach a chemically defined medium,

because it does not necessarily require that a chemically defined medium be used. For example, even if a serum supplement were not used in a medium, other medium supplements, such as a tissue extract, a cell extract, or a hydrolysate, could contain unknown, and thus undefined, ingredients, such that the medium would not be chemically defined.

Further, to rely on an inherency argument, "the examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic *necessarily* flows from the teachings of the applied prior art." *Ex parte Levy*, 17 USPQ2d 1461, 1464 (PTO Bd. Pat. App. Int. 1990); *see also* MPEP § 2112. Here, the Examiner has not provided any basis in fact or technical reasoning to reasonably support the allegation that the medium disclosed in Israel is chemically defined.

Inherent anticipation has not been established. Israel fails to teach the methods of claims 1-29, 79, 140, 154 and 158.

C. Israel Would Not Have Suggested The Methods Of Claims 1-29, 79, 140, 154 and 158

1. A Prima Facie Case Of Obviousness Has Not Been Established

A *prima facie* case of obviousness has not been established. Israel would not have suggested the methods of claims 1-29, 79, 140, 154 and 158 to one of ordinary skill in the art.

A suggestion to modify the teachings in Israel, a reasonable expectation of success in obtaining the claimed methods, and a teaching or suggestion of each element of each claim are required to establish a *prima facie* case of obviousness. *See* MPEP § 2142.

Israel would not have suggested the methods of claims 1-29, 79, 140 and 154, because Israel would not have led one of ordinary skill in the art to use a chemically defined medium. Further, based on Israel, one of ordinary skill in the art would not have had a reasonable expectation of success in cultivating mammalian cells in suspension *in vitro*, using a chemically defined cell culture medium. Moreover, Israel would not have suggested each claim element, because Israel would not have suggested using a chemically defined medium. At best, Israel provides merely an invitation to experiment, not a reasonable expectation of success.

In addition, Israel would not have suggested the method of claim 8, because Israel fails to suggest a 10X concentrated medium formulation. Israel would not have suggested the methods of claims 11, 21 and 29, because Israel fails to suggest a medium containing one or more rice or soy peptides. Israel would not have suggested the method of claim 13, because Israel fails to suggest a medium containing one or more vitamins.

Israel would not have suggested the method of claim 158. At column 1, lines 54-56, Israel discloses that "addition of animal-origin proteins always presents the risk of harboring viruses and other deleterious agents." However, that disclosure by Israel would not have led one of ordinary skill in the art to use a serum-free, non-animal derived cell culture medium, because Israel does not preclude the use of non-protein ingredients of animal origin, such as animal-derived lipids. Further, Israel would not have provided a reasonable expectation of success in cultivating mammalian cells in suspension *in vitro*, using a serum-free, non-animal derived cell culture medium.

2. *It Is Not Applicants' Burden To Establish Non-Obviousness By Objective Evidence, Because A *Prima Facie* Case Of Obviousness Has Not Been Established*

At page 4 of the Office Action, the Examiner stated that "the burden of establishing non-obviousness by objective evidence is shifted to the Applicants," because "the Patent and Trademark Office is not equipped to conduct experimentation in order to determine whether Applicants' claimed methods(s) differ(s) and, if so, to what extent, from the discussed reference [Israel]." Applicants respectfully disagree.

Only if a *prima facie* case of obviousness had been established by an examiner does the burden shifts to Applicants to rebut the *prima facie* case of obviousness with objective evidence of non-obviousness. *See* MPEP § 2112. Here, a *prima facie* case of obviousness has not been established. Accordingly, the burden has not shifted to Applicants to establish non-obviousness by objective evidence.

D. *Summary*

Israel fails to either teach or suggest the methods of claims 1-29, 79, 140, 154 and 158. Applicants respectfully request that these rejections be reconsidered and withdrawn.

VII. *The Rejection Of Claims 30-37 Over Israel and WO 92/05246 Must Be Withdrawn*

At page 4 of the Office Action, the Examiner rejected claims 30-37, under 35 U.S.C. § 103(a), as allegedly obvious over Israel in view of Ramos *et al.*, WO 92/05246

("WO 92/05246"). Applicants respectfully traverse this rejection. A *prima facie* case of obviousness has not been established. Even in combination, Israel and WO 92/05246 would not have suggested the method of claims 30-37.

Claims 1, 15 and 22 recite a method of cultivating a mammalian cell in suspension *in vitro*, using a chemically defined cell culture medium comprising at least one polyanionic or polycationic compound, and claims 1 and 22 recite that the medium is also serum-free. Claims 30-37 depend, either directly or indirectly, from any one of claims 1, 15 or 22.

As discussed above, Israel fails to teach, either explicitly or inherently, cultivation of a cell in suspension *in vitro*, using a chemically defined cell culture medium comprising at least one polyanionic or polycationic compound. WO 92/05246 fails to cure the deficiency of Israel. At page 4, lines 7-10, WO 92/05246 teaches that yeast hydrolysate be used. Yeast hydrolysate is not a chemically defined mixture. Therefore, a medium containing yeast hydrolysate is not a chemically defined medium.

One of ordinary skill in the art would not have been motivated to combine Israel and WO 92/05246 in an effort to obtain a method of culturing cells in a chemically defined medium, because Israel and WO 92/05246 would have suggested neither a chemically defined medium, nor the benefits obtained by using a chemically defined medium. Moreover, even in combination, Israel and WO 92/05246 would not have provided any expectation of success in obtaining a chemically defined medium.

At page 5 of the Office Action, the Examiner stated:

In response to Applicants' argument that a chemically defined culture medium is not disclosed by Israel which is not deemed persuasive because the limitation is inherent to the teachings of Israel, it is also noted that the fact that applicant

has recognized another advantage which would flow naturally from following the suggestion of the prior art cannot be the basis for patentability when the differences would otherwise be obvious.

Applicants respectfully disagree. As discussed above, Israel does not inherently teach a chemically defined medium. Moreover, there is no such thing as "inherent obviousness."

See *In re Spormann and Heinke*, 150 USPQ 449, 452 (CCPA 1966) ("As we pointed out in *In re Adams*, 53 CCPA 966, 356 F.2d 998, 148 USPQ 742, the inherency of an advantage and its obviousness are entirely different questions. That which may be inherent is not necessarily known. Obviousness cannot be predicated on what is unknown.").

Even in combination, Israel and WO 92/05246 would not have suggested a method of cultivating cells using a serum-free, chemically defined medium. Therefore, Israel and WO 92/05246 would not have suggested the method of claims 30-37. Applicants respectfully request that this rejection be reconsidered and withdrawn.

VIII. The Rejection Of Claims 73-77 and 79-82 Over Israel, WO 92/05246 and Inlow Must Be Withdrawn

At page 5 of the Office Action, the Examiner rejected claims 73-77 and 79-82, under 35 U.S.C. § 103(a), as allegedly obvious over Israel in view of WO 92/05246 and Inlow, U.S. Patent Number 5,024,947 ("Inlow"). Applicants respectfully traverse this rejection. A *prima facie* case of obviousness has not been established. Even in combination, Israel, WO 92/05246 and Inlow would not have suggested the method of claims 73-77 and 79-82.

A. A Prima Facie Case Of Obviousness Has Not Been Established

Claim 73 is directed to a method of producing a virus. Claim 79 is directed to a method of producing a polypeptide. Claims 73 and 79 depend multiply from any one of claims 1, 15 or 22. Claims 1, 15 and 22 recite methods of cultivating a mammalian cell in suspension *in vitro*, using a chemically defined cell culture medium comprising at least one polyanionic or polycationic compound, and claims 1 and 22 recite that the medium is also serum-free. Claims 74-77 depend, either directly or indirectly, from claim 73. Claims 80-82 depend, either directly or indirectly, from claim 79.

As discussed above, Israel fails to teach, either explicitly or inherently, cultivation of a cell in suspension *in vitro*, using a chemically defined cell culture medium comprising at least one polyanionic or polycationic compound. WO 92/05246 fails to cure the deficiency of Israel. At page 4, lines 7-10, WO 92/05246 teaches that yeast hydrolysate be used. Yeast hydrolysate is a non-chemically defined mixture.

Likewise, Inlow fails to cure the deficiency of Israel and WO 92/05246, because Inlow fails to teach a chemically defined serum-free medium.

One of ordinary skill in the art would not have been motivated to combine Israel, WO 92/05246, and Inlow in an effort to obtain a method of culturing cells in a chemically defined medium, because Israel, WO 92/05246 and Inlow would have suggested neither a chemically defined medium, nor the benefits obtained from using a chemically defined medium.

Further, one of ordinary skill in the art would not have been motivated to combine Inlow with Israel and WO 92/05246, because Inlow relates only to the culture of insect cells, not mammalian cells. There is no assurance in Israel, WO 92/05246, or Inlow that a serum-

free medium that facilitates production of virus in insect cells would facilitate the production of virus in mammalian cells.

Moreover, even in combination, Israel, WO 92/05246 and Inlow would not have provided a reasonable expectation of success in obtaining a chemically defined medium that facilitates the production of a virus or a polypeptide in mammalian cells. Even in combination, Israel, WO 92/05246 and Inlow would not have suggested a method of producing virus or a polypeptide in mammalian cells using a chemically defined medium.

B. "Serum-Free" Does Not Equate With "Chemically Defined"

At page 5 of the Office Action, the Examiner stated that "in order for a culture medium to be serum free its components must be known and such media would suggest a chemically defined culture medium." Applicants respectfully disagree. A medium can be serum-free, and yet still contain undefined components, *e.g.*, cell or tissue hydrolysates, that are not from serum. Moreover, even if certain known ingredients were included in a medium, other medium components could contain unknown (and thus undefined) ingredients, such that the medium would not be chemically defined.

C. Insect Cells Are Not Predictive Of Mammalian Cells

At pages 5-6 of the Office Action, the Examiner stated that "insect cells are considered to be animal cells and one of skill would have expected successful results cultivating in vitro cells of both mammalian and insectal origin on similar culture medium." Applicants respectfully disagree. One of ordinary skill in the art would not have reasonably expected

successful results culturing mammalian cells, based on Inlow's teaching of an insect cells medium, because there are many differences between cultured mammalian cells and cultured insect cells.

1. *The Growth Temperature For Culturing Mammalian Cells Is Different Than The Growth Temperature For Culturing Insect Cells*

Attachment 1 is Weiss *et al.*, "Serum-Free Media," in: *Insect Cell Culture Engineering*, M.F.A. Goosen and A.J. Daugulis, Eds., Marcel Dekker, Inc., publ., pp. 179-195 (1993) ("Weiss").¹ Weiss teaches that, whereas the optimal temperature for culturing mammalian cells is 37°C, the optimal temperature for culturing insect cells is 28°C. See Weiss at 181, Table 1.

Attachment 2 is GibcoBRL - Guide to Baculovirus Expression Vector Systems (BEVS) and Insect Cell Culture Techniques ("GibcoBRL"), which teaches at page 5 that the optimal temperature for culturing insect cells is from 25-30°C.²

Moreover, Inlow teaches that the optimal temperature range for culturing *Spodoptera frugiperda* (insect) cells is 25-32°C. See Inlow at column 17, lines 60-63.

¹ Weiss is co-authored by Glenn P. Godwin and Stephen F. Gorfien, who are co-inventors of subject matter claimed in the present application. Each of the authors of Weiss either are now or were employed at GIBCO BRL Cell Culture R&D, which is a division of Life Technologies, Inc., the original assignee of this application. Invitrogen Corporation became the owner of the above-captioned application by virtue of a merger on September 14, 2000, with Life Technologies, Inc.

² This document was published by Life Technologies, Inc., the original assignee of this application.

Thus, the growth temperature requirement for culturing mammalian cells is significantly different from the temperature requirement for culturing insect cells.

2. *The pH Requirement For Culturing Mammalian Cells Is Different Than The pH Requirement For Culturing Insect Cells*

Attachment 3 is "The Culture Environment: Substrate, Gas Phase, Medium, and Temperature," in: *CULTURE OF ANIMAL CELLS: A Manual Of Basic Technique*, R.I. Freshney, Alan R. Liss, Inc., publ., pp 57-84 (1987) ("Freshney"). Freshney teaches at page 69 that the optimal pH for most cell lines of animal origin is about 7.4.

Inlow teaches that the optimal pH range for culturing *Spodoptera frugiperda* (insect) cells is from 6 to 7. See Inlow at column 17, lines 63-64. GibcoBRL teaches at page 5 that the optimal temperature for culturing lepidopteran (insect) cells is from 6.0 to 6.4.

Thus, the pH requirement for culturing mammalian cells is significantly different from the pH requirement for culturing insect cells.

3. *The Osmolality Requirement For Culturing Mammalian Cells Is Different Than The Osmolality Requirement For Culturing Insect Cells*

Freshney teaches at page 70 that the optimal osmolality range for culturing animal cells is from 260 to 320 mOsm/kg.

GibcoBRL teaches at page 5 that the optimal osmolality range for lepidopteran (insect) cell lines is 345 to 380 mOsm/kg. Thus, the osmolality requirement for culturing mammalian cells is significantly different from the osmolality requirement for culturing insect cells.

4. *Other Differences Between Culturing Mammalian Cells And Insect Cells*

Weiss teaches that there are several other differences between the requirements for culturing mammalian cells and insect cells. Although growth of insect cells in serum-free medium was considered easy, growth of mammalian cells in serum-free medium was considered difficult. *See* Weiss at 181, Table 1.

Although growth of insect cells to high density was considered easy, growth of mammalian cells to high density was considered difficult. *See* Weiss at 181, Table 1.

Although mammalian cell culture is dependent on carbon dioxide, insect cell culture is not. *See* Weiss at 181, Table 1.

Although maintenance of insect cells in culture was considered easy, maintenance of mammalian cells in culture was considered difficult. *See* Weiss at 181, Table 1.

Although mammalian cells were considered susceptible to changes in pH and osmotic pressure, insect cells were not. *See* Weiss at 181, Table 1.

5. *Conclusion*

For the above reasons, the growth requirements for insect cells are completely different than the growth requirements for mammalian cells. A medium that supports insect cell culture would not have been reasonably expected to support mammalian cell culture.

D. *Summary*

A *prima facie* case of obviousness has not been established over Israel, WO 92/05246 and Inlow. Applicants respectfully request that this rejection be reconsidered and withdrawn.

IX. The Rejection Of Claims 106 and 143-149, 155 and 157 Over Keen Must Be Withdrawn

At page 6 of the Office Action, the Examiner rejected claims 106, 143-149, 155 and 157, under 35 U.S.C. § 102(b), as allegedly anticipated by Keen, U.S. Patent Number 5,316,938 ("Keen"), or, in the alternative, under 35 U.S.C. § 103(a), as allegedly obvious over Keen. Applicants respectfully traverse these rejections.

A. Keen Fails To Teach Or Suggest The Methods Of Claims 106, 143-149, 155 and 157

Claim 106 recites a method of cultivating mammalian cells in suspension culture and/or expressing a recombinant protein, using a cell culture medium comprising an iron chelate and a zinc salt, and that does not contain insulin.

Keen relates to a culture medium for culturing engineered CHO cells. Keen fails to anticipate claims 106, 143-149, 155 and 157, because Keen teaches that the medium *must* include a growth factor.

The present invention therefore provides a biochemically defined culture medium for culturing engineered CHO cells . . . comprising water . . . an inorganic or recombinant iron source, and a recombinant or synthetic growth factor and *optionally* non-ferrous metal ions, vitamins and cofactors.

Keen at column 3, lines 2-11 (emphasis added). Had addition of a growth factor been optional, Keen would have indicated such, because Keen knew how to indicate whether addition of an ingredient is optional. *See* Keen at column 3, line 10.

Keen also teaches that such growth factors include insulin. *See* Keen at column 5, lines 36-43. Further, the only two media exemplified by Keen each contain insulin. *See* Keen at column 7, line 61; and column 8, line 23.

With respect to the rejection of claim 106 for alleged anticipation, Keen fails to teach claim 106, because Keen fails to teach a medium that does not contain insulin.

With respect to the rejection of claim 106 for alleged obviousness, a *prima facie* case of obviousness has not been established. Keen would not have suggested a medium that does not contain insulin. Indeed, by teaching that insulin be included in the medium, Keen teaches away from the method claim 106.

Claims 143-149 and 155 depend from claim 106. Keen fails to teach or suggest the method of claims 143-149 and 155 for the same reasons that Keen fails to teach or suggest the method of claim 106.

Keen also fails to teach or suggest the method of claim 143, because Keen fails to teach or suggest a medium that is free of animal-derived ingredients.

Keen also fails to teach or suggest the method of claim 144, because Keen fails to teach or suggest a medium that is protein-free.

Keen also fails to teach or suggest the method of claim 145, because Keen fails to teach or suggest a medium that is does not include insulin, and that is chemically defined.

Keen also fails to teach or suggest the method of claim 146, because Keen fails to teach or suggest a medium that contains neither transferrin nor insulin.

Keen also fails to teach or suggest the method of claim 147, because Keen fails to teach or suggest a medium that does not include insulin, and that supports the growth of CHO cells.

Keen also fails to teach or suggest the method of claim 148, because Keen fails to teach or suggest a 1X medium formulation that does not include insulin.

Keen also fails to teach or suggest the method of claim 149, because Keen fails to teach or suggest a concentrated medium formulation that does not include insulin.

Keen also fails to teach or suggest the method of claim 155, because Keen fails to teach or suggest a serum-free medium that does not contain insulin and that supports high-density growth of cells.

C. Keen Fails To Teach Or Suggest The Method of Independent Claim 157

Claim 157 is directed to a method for replacing protein in a mammalian cell culture medium, the method comprising replacing insulin with a Zn^{2+} salt and replacing transferrin with a Fe^{2+} chelate and/or a Fe^{3+} chelate. Keen fails to teach or suggest replacing insulin with a Zn^{2+} salt and replacing transferrin with a Fe^{2+} chelate and/or a Fe^{3+} chelate.

D. Summary

Keen fails to teach the methods of claims 106, 143-149, 155 and 157. Moreover, a *prima facie* case of obviousness has not been established. Applicants respectfully request that these rejections be withdrawn.

X. The Rejection Of Claims 150-153 Over Keen Must Be Withdrawn

At page 6 of the Office Action, the Examiner rejected claims 150-153, under 35 U.S.C. § 103(a), as allegedly obvious over Keen. Applicants respectfully traverse this rejection. A *prima facie* case of obviousness has not been established. Keen teaches away from the claimed method.

Claims 150 and 151 depend from claim 149, and claim 149 depends from claim 106. Claims 152 and 152 depend, either directly or indirectly, from claim 106. Claim 106 recites a method of cultivating mammalian cells in suspension culture and/or expressing a recombinant protein, using a cell culture medium comprising an iron chelate and a zinc salt, and that does not contain insulin.

At pages 6-7 of the Office Action, the Examiner stated that "Keen et al. is argued by Applicants as allegedly explicitly teaching the requirement for insulin, however, at Table 1, columns 4-5, all lines, not [sic] insulin is disclosed in the medium A and it is thus, not explicitly taught as argued by Applicants." Applicants respectfully disagree. Table 1 in Keen provides a basal medium, to which other ingredients are to be added. Thus, the basal medium in Table 1 is not the medium that Keen teaches be used to culture cells. Keen teaches that medium A be supplemented with insulin. *See* Keen at column 7, line 61; and column 8, line 23.

At page 7 of the Office Action, the Examiner stated that "to vary concentrations of chelators is clearly within the purview of an ordinary artisan as is the use of various media for cultivation of cells." Applicants respectfully disagree. The standard for obviousness is *not* whether claimed subject matter is allegedly "within the purview" of the artisan. As the MPEP provides:

A statement that modifications of the prior art to meet the claimed invention would have been "*well within the ordinary skill of the art at the time the claimed invention was made*" because the references relied upon teach that all aspects of the claimed invention were individually known in the art is not sufficient to establish a *prima facie* case of obviousness without some objective reason to combine the teachings of the references.

MPEP § 2143.01, page 2100-99 (Feb. 1, 2000) (citation omitted). Here, the Examiner has done what the MPEP explicitly instructs should not be done. No objective reason for relying on Keen to suggest the claimed method of claims 150-153 has been provided. Thus, the basis for this rejection is improper.

A suggestion to modify the teachings in Israel, a reasonable expectation of success in obtaining the claimed methods, and a teaching or suggestion of each element of each claim are required to establish a *prima facie* case of obviousness. See MPEP § 2142.

For Keen to have provided motivation, Keen must have led one of ordinary skill in the art to obtain the claim invention. Here, Keen would not have led one of ordinary skill in the art to the medium of the methods of claims 150-153, because Keen teaches explicitly that a medium must contain insulin. Thus, Keen teaches away from the claimed method.

For Keen to have provided a reasonable expectation of success, Keen would had to have predicted that a medium devoid of insulin would have supported the growth of cells and/or the expression of recombinant protein. Given the explicit teaching in Keen to use insulin, Keen could not have provided the artisan with any expectation of success in obtaining a medium that does not contain insulin and that would have supported the growth of cells and/or the expression of recombinant protein.

Thus, Keen would not have suggested the method of claim 106. Since Keen would not suggested the method of claim 106, Keen would not have suggested the method of claim 150, in which a 10X medium formulation is recited, or the method of claim 151, in which a medium formulation of greater than 10X is recited.

Keen would also not have suggested the method of claim 152, because Keen would not have suggested the desirability of the specific range of iron and the specific range of zinc that are recited in claim 152.

Keen would also not have suggested the method of claim 153, because Keen would not have suggested the desirability of the specific concentration of iron and the specific concentration of zinc that are recited in claim 153.

Applicants respectfully request that this rejection be reconsidered and withdrawn.

X. *The Rejection Of Claims 106-112, 143-153, 156 and 157 Over Keen, Israel and Inlow Must Be Withdrawn*

At page 7 of the Office Action, the Examiner rejected claims 106-112, 143-153, 156 and 157, under 35 U.S.C. § 103(a), as allegedly obvious over Keen in view of Israel and Inlow. Applicants respectfully traverse this rejection. A *prima facie* case of obviousness has not been established. Even in combination, Keen, Israel and Inlow would not have suggested the methods of claims 106-112, 143-153, 156 and 157.

Claims 107-112, 143-153 and 156 depend, either directly or indirectly, from claim 106. Claim 106 recites a method of cultivating mammalian cells in suspension culture and/or expressing a recombinant protein, using a cell culture medium comprising an iron chelate and a

zinc salt, and that does not contain insulin. Claim 157 recites method for replacing protein in a mammalian cell culture medium, comprising replacing insulin with a Zn^{2+} salt and replacing transferrin with a Fe^{2+} chelate and/or replacing transferrin with a Fe^{3+} chelate.

As discussed above, Keen teaches away from the claimed methods, because Keen teaches that insulin is necessary. Israel and Inlow fail to cure the deficiency of Keen, because neither Israel nor Inlow would have suggested that a medium comprising an iron chelate and a zinc salt, and that does not contain insulin, would support cultivation of mammalian cells in suspension culture and/or expression of a recombinant protein. Further, Inlow relates only to insect cells, not to mammalian cells. As discussed above, Inlow would not have suggested that a medium that supports insect cell culture would support mammalian cell culture.

Moreover, Applicants have discovered that, in a chemically defined medium, transferrin can be replaced by an iron chelate, and insulin can be replaced by a zinc salt. Keen, Israel and Inlow would not have suggested either that transferrin can be replaced by an iron chelate, or that insulin can be replaced by a zinc salt.

Therefore, even in combination, Keen, Israel and Inlow would have taught away from the methods of claim 106-112, 143-153 and 156.

Keen, Israel and Inlow would also not have suggested the method of claim 143, because Keen, Israel and Inlow would not have suggested a medium that is free of animal-derived ingredients.

Keen, Israel and Inlow would also not have suggested the method of claim 144, because Keen, Israel and Inlow would not have suggested a medium that is protein-free.

Keen, Israel and Inlow would also not have suggested the method of claim 145, because Keen, Israel and Inlow would not have suggested a medium that does not contain insulin and that is chemically defined.

Keen, Israel and Inlow would also not have suggested the method of claim 146, because Keen, Israel and Inlow would not have suggested a medium that contains neither transferrin nor insulin.

Keen, Israel and Inlow would also not have suggested the method of claim 147, because Keen, Israel and Inlow would not have suggested a medium that does not include insulin, and that supports the growth of CHO cells.

Keen, Israel and Inlow would also not have suggested the method of claim 148, because Keen, Israel and Inlow would not have suggested a 1X medium formulation that does not include insulin.

Keen, Israel and Inlow would also not have suggested the method of claim 149, because Keen, Israel and Inlow would not have suggested a concentrated medium formulation that does not include insulin.

Keen, Israel and Inlow would also not have suggested the method of claim 150, in which a 10X medium formulation is recited, or the method of claim 151, in which a medium formulation of greater than 10X is recited.

Keen, Israel and Inlow would also not have suggested the method of claim 152, because Keen, Israel and Inlow would not have suggested the desirability of the specific range of iron and the specific range of zinc that are recited in claim 152.

Keen, Israel and Inlow would also not have suggested the method of claim 153, because Keen, Israel and Inlow would not have suggested the desirability of the specific concentration of iron and the specific concentration of zinc that are recited in claim 153.

Keen, Israel and Inlow would also not have suggested the method of claim 156, because Keen, Israel and Inlow would not have suggested a medium that supports high density growth of CHO cells.

Keen, Israel and Inlow would also not have suggested the method of claim 157, because Keen, Israel and Inlow would not have suggested replacing insulin with a Zn^{2+} salt and replacing transferrin with a Fe^{2+} chelate and/or replacing transferrin with a Fe^{3+} chelate.

Applicants respectfully request that this rejection be reconsidered and withdrawn.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed. Applicants therefore respectfully request that the Examiner reconsider and withdraw all of the presently outstanding rejections. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully
requested.

Respectfully submitted,

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Version with markings to show changes made

152. (Amended) The method of claim 106, wherein [the concentration of Fe^{2+} or Fe^{3+} is] Fe^{2+} and/or Fe^{3+} is present at a concentration of about 0.00028 to 0.011 g/L and the concentration of Zn^{2+} is about 0.00007 to 0.00073 g/L.

153. (Amended) The method of claim 152, wherein the concentration of Fe^{2+} and/or Fe^{3+} is about 0.0011 g/L and the concentration of Zn^{2+} is about 0.000354 g/L.

157. (Amended) A method for replacing protein in a mammalian cell culture medium, said method comprising
replacing insulin with a Zn^{2+} salt and [and/or] replacing transferrin with a Fe^{2+} chelate and/or [replacing transferrin with] a Fe^{3+} chelate.